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Identification of the naturally occurring genes encoding carbapenem-hydrolysing oxacillinases from *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, and *Acinetobacter calcoaceticus*

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Abstract

Carbapenem resistance is increasingly being reported among *Acinetobacter* species, and results mostly from the expression of acquired carbapenem-hydrolysing oxacillinases (CHDLs). Several *Acinetobacter* species intrinsically possess chromosomal CHDL genes: *Acinetobacter baumannii* (bla_{OXA-51}), *Acinetobacter radioresistens* (bla_{OXA-23}), and *Acinetobacter lwoffii* ($bla_{OXA-134}$). We aimed to identify the progenitors of novel CHDL-encoding genes for identification of potential reservoirs. We performed PCR screening using degenerated internal primers designed from a sequence alignment of the known CHDLs (OXA-23, OXA-40, OXA-51, OXA-58, OXA-134, and OXA-143) applied to a collection of 50 *Acinetobacter* strains belonging to 23 different species. Two strains of *Acinetobacter johnsonii*, one strain of *Acinetobacter calcoaceticus* and two strains of *Acinetobacter haemolyticus* were found to harbour, respectively, the totally novel $bla_{OXA-211}$-like, $bla_{OXA-213}$-like and $bla_{OXA-214}$-like genes. In addition, the complete genomes of those three species available in GenBank, i.e. one *A. johnsonii* genome, four *A. calcoaceticus* genomes, and one *A. haemolyticus* genome, were analysed and found to be positive for the presence of bla_{OXA211}-like, $bla_{OXA-213}$-like and $bla_{OXA-214}$-like genes, respectively. The β-lactamases OXA-211, OXA-213 and OXA-214 are diverse, with amino acid identities ranging from 53% to 76%, as compared with the naturally occurring OXA-51-like CHDL from *A. baumannii*. These β-lactamases showed a peculiar hydrolysis profile, including mostly penicillins and carbapenems. Regarding bla_{OXA-23} in *A. radioresistens* and $bla_{OXA-134}$ in *A. lwoffii*, these genes were not expressed (or expressed at a non-significant level) in their host. Detection of these β-lactamase genes might be used as a useful tool for accurate identification of these *Acinetobacter* species.

Keywords: Carbapenem resistance, CHDL, Gram-negative rods, specific activity
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Introduction

Although *Acinetobacter baumannii* is considered to be the most clinically relevant *Acinetobacter* species, there are increasing reports of community and nosocomial infections caused by non-*baumannii Acinetobacter* species [1]. This is probably a consequence of the development of genetic tools for identification of the different *Acinetobacter* species. *Acinetobacter haemolyticus* was found to be a causative agent of endocarditis [2] and bloody diarrhoea mediated by Shiga toxin production [3]. *Acinetobacter johnsonii* was found to be associated with catheter-related bloodstream infections [4]. As there is a close relationship between *A. baumannii* and *Acinetobacter calcoaceticus*, the term *A. baumannii–A. calcoaceticus* complex, or Abc complex, has been used, and the exact clinical relevance of *A. calcoaceticus* therefore remains unclear [5]. Although antimicrobial susceptibility in all other *Acinetobacter* species is usually much higher than in *A. baumannii*, antimicrobial resistance in non-*baumannii Acinetobacter* species raises concern, as multidrug-resistant isolates, including carbapenem-resistant isolates, are being reported worldwide [6,7]. Furthermore, some

Acinetobacter species have been shown to intrinsically possess chromosomal genes encoding carbapenem-hydrolysing oxacillinases (CHDLs). Thus, *A. baumannii* carries bla_{OXA-51}-like, *Acinetobacter radioresistens* bla_{OXA-23}-like and *Acinetobacter lwoffii* $bla_{OXA-134}$-like genes [8–10]. Characterization of these naturally occurring CHDL-encoding genes seems important for two reasons. First, these genes may spread into other *Acinetobacter* species, such as *A. baumannii*, causing outbreaks of carbapenem-resistant strains, as has been reported with bla_{OXA-23}-carrying *A. baumannii* isolates [11]. Second, detection of these β-lactamase genes might be used as an additional tool for rapid identification of *Acinetobacter* species.

Materials and Methods

Bacterial strains

A. haemolyticus VAL was recovered from a clinical sample. *A. calcoaceticus* CIP81.8 and *A. johnsonii* CIP70.16 were purchased from the Institut Pasteur collection. *A. baumannii* AYE and *A. radioresistens* strain 2 were recovered from clinical samples [10]. Our screening panel included *Acinetobacter* strains belonging to 23 different *Acinetobacter* species, including *Acinetobacter junii*, *A. johnsonii*, *A. haemolyticus*, *Acinetobacter baylyi*, *A. lwoffii*, *A. radioresistens*, *Acinetobacter schindleri*, *Acinetobacter ursingii*, *A. calcoaceticus*, *Acinetobacter gerneri*, *Acinetobacter tjernbergiae*, *Acinetobacter bouvetii*, *Acinetobacter tandoii*, *Acinetobacter grimontii*, *Acinetobacter towneri*, *Acinetobacter parvus*, *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3), *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU) [12], *Acinetobacter bereziniae* sp. nov. (formerly *Acinetobacter* genomic species 10) [13], and *Acinetobacter* genomospecies 6, 15, 16, and 17. These strains were identified at the species level with the use of 16S rRNA sequencing [14].

Antimicrobial agents and MIC determinations

The antimicrobial agents and their sources have been described previously [14]. Antibiotic-containing disks were used for detection of antibiotic susceptibility, with Mueller–Hinton agar plates (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France) and a disk diffusion assay (http://www.sfm.fr). MICs were determined by agar dilution and Etest (AB Biodisk, Solna, Sweden) [15], with results interpreted according to the guidelines of the CLSI [16].

PCR amplification and cloning experiments

Whole cell DNA of *Acinetobacter* species was extracted as described previously [10]. PCRs were performed in standard conditions with Taq DNA polymerase (Applied Biosystems, Courtaboeuf, France), as described previously [10]. Screening for CHDL-encoding genes was performed by PCR with the degenerate internal primers OXA-CHDL A and OXA-CHDL B (Table 1). Degenerate internal primers were designed in the corresponding nucleotide regions of conserved protein sequences after complete OXA-23, OXA-40, OXA-58, OXA-51, OXA-134 and OXA-143 protein sequence alignment. Complete available genome sequences of *A. haemolyticus* ATCC 19194 (GenBank accession number NZ_ADMT00000000.1), *A. johnsonii* SH046 (Genbank accession number ACPL01000000) and *A. calcoaceticus* RUH2202 (GenBank accession number ZP_06056651.1) were screened in order to determine genomic sequences flanking the internal fragments of the novel genes temporarily named bla_{OXA-H}, bla_{OXA-J}, and bla_{OXA-C}, respectively. External primers specific for each gene were designed and used for PCR, allowing amplification of the entire sequences of these genes (Table 1). OXA-calcoaceticus A-B, OXA-haemolyticus A-B and OXA-johnsonii A-B (Table 1) were used to amplify fragments containing the entire oxacillinase-encoding gene, with whole cell DNA from *A. calcoaceticus*, *A. haemolyticus* and *A. johnsonii* as the template.

The obtained PCR fragment was purified with a QIAquick column (Qiagen, Courtaboeuf, France) and cloned into the pTOPO vector. Recombinant plasmids were selected on trypticase soy agar plates containing amoxycillin (25 mg/mL) and kanamycin (30 mg/mL). The cloned DNA fragments inserted into one of the recombinant plasmids were sequenced each

TABLE 1. Primers used in this study

Primers	Sequence (5′ to 3′)	Purpose	Expected amplicon size (bp)
OXA-CHDL A	CCHGCHTCDACHTTYAARAT	Detection of CHDL	485
OXA-CHDL B	KYHAYABCCMWKSCCCADCC		
OXA-calcoaceticus A	ACCGCCATATCAACTTTCC	Detection and amplification of natural CHDL of *Acinetobacter calcoaceticus*	1028
OXA-calcoaceticus B	TTGAGCACACCATTTCATCC		
OXA-haemolyticus A	TTTTCTAGCTCGGCTTTCCCC	Detection and amplification of natural CHDL of *Acinetobacter haemolyticus*	1075
OXA-haemolyticus B	ATCCCTCTAGGATGATTTCTCCC		
OXA-johnsonii A	TTTAGATCTTAGCCACCC	Detection and amplification of natural CHDL of *Acinetobacter johnsonii*	863
OXA-johnsonii B	AATACGTCTTCTCTATGGGC		

CHDL, carbapenem-hydrolysing oxacillinase.

time from two clones on both strands with an Applied Biosystems sequencer (ABI 377).

β-Lactamase preparation and biochemical analysis

Cultures of *Escherichia coli* expressing $bla_{OXA-211}$, $bla_{OXA-213}$ and $bla_{OXA-214}$ were grown overnight at 37°C in 100 mL of trypticase soy broth with amoxycillin (25 mg/L). Bacterial suspensions were disrupted by sonication (twice for 30 s each time at 20 Hz (Phospholyser Vibra Cell 300; Bioblock, Illkirch, France)) and centrifuged (10 min, 10 000 ***g***, 4°C). The supernatants contained the crude enzyme extracts. Crude enzyme extracts containing partially purified β-lactamases were subjected to specific activity measurements. The β-lactamase activities of *E. coli* TOP10 cultures harbouring pOXA-211, pOXA-213 and pOXA-214 were assayed by UV spectrophotometry (Spectrophotometer Ultrospec 2000; Pharmacia Biotech, Orsay, France) at 30°C in 100 mM phosphate buffer (pH 7.0), as previously described [17].

Dendrogram and protein analysis

The nucleotide and deduced amino acid sequences were analyzed and compared with sequences available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

Screening of CHDL-encoding genes in a collection of *Acinetobacter* species.

PCR screening with degenerate internal primers gave positive results for *A. baumannii*, *A. radioresistens*, *A. lwoffii*, *A. haemolyticus*, *A. johnsonii*, and *A. calcoaceticus*. After sequencing, *A. baumannii*, *A. radioresistens* and *A. lwoffii* were found to possess the bla_{OXA-51}, bla_{OXA-23} and $bla_{OXA-134}$ genes, respectively, in accordance with previous results [8–10]. PCR screening with these degenerate internal primers gave weak

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OXA-211     ---MKTLQLALIALITTFGSACTTIPPSVETAKNHQQQSAQQQIQQAFDQLQTTGVIVIK
OXA-213     ---MYKKALIVATSILFLSACSSNMVKQHQIHSIS-ANKNSEEIKSLFDQAQTTGVLVIK
OXA-214     --MKLSKLYTLTVLIGFGLSGVACQHIHTPVSFNQIENDQTKQIASLFENVQTTGVLITF
OXA-51      ---MNIKTLLLITSAIFISACSPYIVTANPNHSASKSDEKAEKIKNLFNEVHTTGVLVIQ
OXA-40      -MKKFILPIFSISILVSLSACSSIKTKSEDNFHIS-SQQHEKAIKSYFDEAQTQGVIIIK
OXA-23      ---MNKYFTCYVVASLFLSGCTVQHNLIN-ETPSQIVQGHNQVIHQYFDEKNTSGVLVIQ
OXA-58      MKLLKILSLVCLSISIGACAEHSMSRAKTSTIPQVNNSIIDQNVQALFNEISADAVFVTY

                                70  73
                                 |  |
OXA-211     DKHGLHSYGNDLSRAQTPYVPASTFKMLNALIGLEHGKATSTEVFKWDGQKRSFPAWEKD
OXA-213     RGQTEEIYGNDFKRASTDYVPASTFKMLNALIGLEHHKATTTEVFKWNGQKRLFPDWEKD
OXA-214     DGQAYKAYGNDLNRAKTAYIPASTFKILNALIGIEHDKTSPNEVFKWDGQKRAFESWEKD
OXA-51      QGQTQQSYGNDLARASTEYVPASTFKMLNALIGLEHHKATTTEVFKWDGQKRLFPEWEKD
OXA-40      EGKNLSTYGNALARANKEYVPASTFKMLNALIGLENHKATTNEIFKWDGKKRTYPMWEKD
OXA-23      TDKKINLYGNALSRANTEYVPASTFKMLNALIGLENQKTDINEIFKWKGEKRSFTAWEKD
OXA-58      DGQNIKKYGTHLDRAKTAYIPASTFKIANALIGLENHKATSTEIFKWDGKPRFFKAWDKD

OXA-211     MTLGQAMQASAVPVYQELARRIGLDLMKKEVQRIGYGNQQIGTVVDNFWLVGPLQITPVQ
OXA-213     MTLGDAMKASAIPVYQELARRIGLDLMSKEVKRVGFGNANIGSKVDNFWLVGPLKITPQQ
OXA-214     LTLAEAMQASAVPVYQALAQRIGLDLMAKEVKRVGFGNTRIGTQVDNFWLIGPLKITPIE
OXA-51      MTLGDAMKASAIPVYQDLARRIGLELMSKEVKRVGYGNADIGTQVDNFWLVGPLKITPQQ
OXA-40      MTLGEAMALSAVPVYQELARRTGLELMQKEVKRVNFGNTNIGTQVDNFWLVGPLKITPVQ
OXA-23      MTLGEAMKLSAVPVYQELARRIGLDLMQKEVKRIGFGNAEIGQQVDNFWLVGPLKVTPIQ
OXA-58      FTLGEAMQASTVPVYQELARRIGPSLMQSELQRIGYGNMQIGTEVDQFWLKGPLTITPIQ

OXA-211     EVLFVEKLANTQLAFKPDVQHTVQDMLLIEQKPNYKLYAKSGWGMDLEPQVGWWTGWVET
OXA-213     ETQFAYQLAHKTLPFSKDVQEQVQSMVFIEEKNGSKIYAKSGWGWDVEPQVGWLTGWVVQ
OXA-214     EAQFAYRLAKQELPFTPKTQQQVIDMLLVDEIRGTKVYAKSGWGMDITPQVGWWTGWIED
OXA-51      EAQFAYKLANKTLPFSPKVQDEVQSMLFIEEKNGNKIYAKSGWGWDVDPQVGWLTGWVVQ
OXA-40      EVNFADDLAHNRLPFKLETQEEVKKMLLIKEVNGSKIYAKSGWGMGVTPQVGWLTGWVEQ
OXA-23      EVEFVSQLAHTQLPFSEKVQANVKNMLLLEESNGYKIFGKTGWAMDIKPQVGWLTGWVEQ
OXA-58      EVKFVYDLAQGQLPFKPEVQQQVKEMLYVERRGENRLYAKSGWGMAVDPQVGWYVGFVEK

OXA-211     ATSEKVYFALNMHMKTGISASVREQLVKQSLTALGII---
OXA-213     PQGEIVAFSLNLEMKKGTPSSIRKEIAYKGLEQLGIL---
OXA-214     PNGKVIAFSLNMEMNQPAHAAARKEIVYQALTQLKLL---
OXA-51      PQGNIVAFSLNLEMKKGIPSSVRKEITYKSLEQLGIL---
OXA-40      ANGKKIPFSLNLEMKEGMSGSIRNEITYKSLENLGII---
OXA-23      PDGKIVAFALNMEMRSEMPASIRNELLMKSLKQLNII---
OXA-58      ADGQVVAFALNMQMKAGDDIALRKQLSLDVLDKLGVFHYL
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FIG. 1. Amino acid alignment of β-lactamases OXA-211, OXA-213 and OXA-214 identified in this study from *Acinetobacter johnsonii*, *Acinetobacter calcoaceticus*, and *Acinetobacter haemolyticus*, respectively. Amino acid motifs that are well conserved (even if possibly variable) among class D β-lactamases (DBLs) are shaded in grey [19]. Numbering is according to DBL numbering [16]. Asterisks indicate identical amino acids between all sequences.

bands for the following isolates: *A. bouvettii*, *A. parvus*, *A. pittii* sp. nov., *A. nosocomialis* sp. nov., *A. bereziniae* sp. nov., *Acinetobacter* genomic species 6, and *Acinetobacter* genomic species 17. The corresponding sequences were relatively far from the sequences of the known CHDL-encoding genes from *Acinetobacter* species: bla_{OXA-23}, bla_{OXA-40}, bla_{OXA-51}, bla_{OXA-58}, and $bla_{OXA-134}$ (data not shown). Thus, these weakly positive PCR results were not further investigated. PCR screening was negative for *A. junii*, *A. baylyi*, *A. schindleri*, *A. ursingii*, *A. gerneri*, *A. tjernbergiae*, *A. tandoii*, *A. grimontii*, and *A. towneri*.

Sequencing of CHDL-encoding genes

Sequencing of the amplicons obtained from *A. haemolyticus*, *A. johnsonii* and *A. calcoaceticus* identified three genes encoding novel OXA-type β-lactamases. OXA-H was named OXA-214, and shared 53% amino acid identity with OXA-23, 55% with OXA-51, 53% with OXA-40, and 48% with OXA-58. OXA-J was named OXA-211, and shared 55% amino acid identity with OXA-23, 58% with OXA-51, 56% with OXA-40, and 52% with OXA-58. OXA-C was named OXA-213, and shared 57% amino acid identity with OXA-23, 76% with OXA-51, 62% with OXA-40, and 46% with OXA-58.

OXA-211, OXA-213 and OXA-214 possessed the typical features of class D β-lactamases (DBLs), including the STFK tetrad at positions 70–73 according to DBL numbering (Fig. 1) [16]. Also, in OXA-213 and OXA-214, an FGN motif at DBL positions 144–146 replaced the usual YGN motif of classical DBLs, as observed for other CHDLs except for OXA-58, OXA-51, and OXA-211 [17]. Finally, a KSG element was identified at DBL positions 216–218, as observed in the CHDLs OXA-40 and OXA-51, whereas a KTG motif is present in most DBLs, including the CHDL OXA-23 [17]. A phylogenetic analysis showed that OXA-211, OXA-213 and OXA-214 constituted separate subgroups of CHDLs, but that these subgroups were more closely related to the identified DBLs from *Acinetobacter* species than to other known CHDLs (Fig. 2).

Susceptibility patterns

All of the *A. haemolyticus* VAL, *A. johnsonii* CIP70.16, *A. calcoaceticus* CIP81.8 and *A. radioresistens* strain 2 isolates were fully susceptible to all antibiotics tested, including penicillins and carbapenems (Table 2). *A. baumannii* AYE was only susceptible to carbapenems, because it carried the extended-spectrum β-lactamase VEB-1 and overexpressed the *ampC* gene, as previously described [18].

Distribution of OXA-211, OXA-213 and OXA-214 CHDL-encoding genes

In order to assess whether the novel OXA-encoding genes were naturally present in the *Acinetobacter* species, the $bla_{OXA-211}$, $bla_{OXA-213}$ and $bla_{OXA-214}$ genes were screened

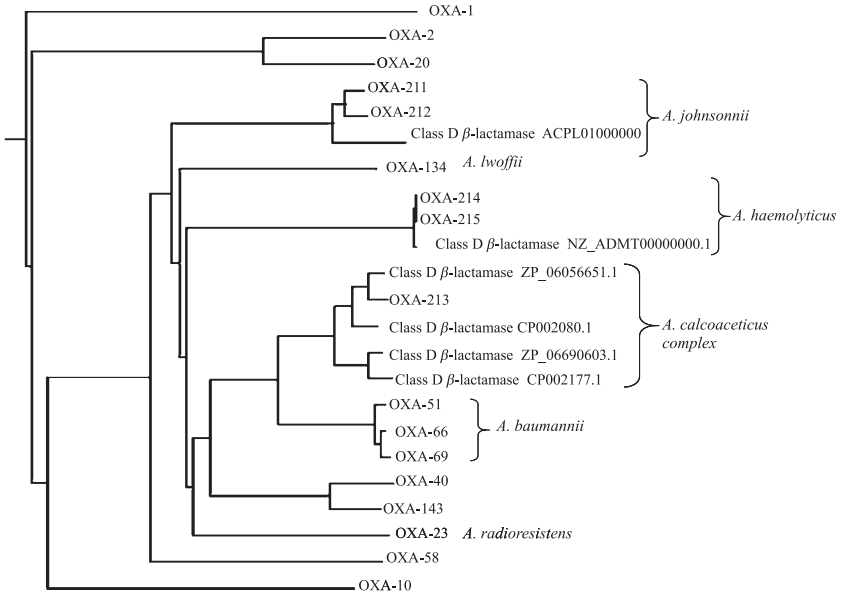

FIG. 2. Dendrogram obtained for 23 class D β-lactamases by neighbour-joining analysis. The alignment used for tree calculation was performed with ClustalX. Branch lengths are drawn to scale, and are proportional to the number of amino acid changes. The distance along the vertical axis has no significance. The class D β-lactamases that are considered to be naturally occurring are indicated, together with the names of the corresponding species.

out in additional clinical isolates of *A. johnsonii*, *A. calcoaceticus*, and *A. haemolyticus*, respectively. PCR with specific primers was performed, and gave positive results for the $bla_{OXA-214}$ gene in the two *A. haemolyticus* isolates tested, for the $bla_{OXA-211}$ gene in the two *A. johnsonii* isolates tested, and for the $bla_{OXA-213}$ gene in the one *A. calcoaceticus* isolate tested.

In addition, the $bla_{OXA-211}$, $bla_{OXA-213}$ and $bla_{OXA-214}$ genes were screened out in all complete genomes of these three species available on Genbank. The $bla_{OXA-211}$ gene was identified in one *A. johnsonii* genome (GenBank accession number ACPL01000000), the $bla_{OXA-214}$ gene was identified in one *A. haemolyticus* genome (GenBank accession number NZ_ADMT00000000.1), and the $bla_{OXA-213}$ gene was identified in four *A. calcoaceticus* genomes (*A. calcoaceticus* RUH2202, GenBank accession number ZP_06056651.1; *A. calcoaceticus* DR1, GenBank accession number CP002080.1; *A calcoaceticus* PHEA-2, GenBank accession number CP002177.1; and *Acinetobacter* sp. SH024, GenBank accession number ZP_06690603.1).

Thus, screening of clinical isolates and analysis of the available complete genomes for each species allowed identification of a total of three variants of OXA-214 from *A. haemolyticus*, three variants of OXA-211 from *A. johnsonii*, and five variants of OXA-213 from *A. calcoaceticus*.

Biochemical properties of OXA-211, OXA-213, OXA-214

To further characterize the activity of OXA-211, OXA-213 and OXA-214, the enzymes were purified from *E. coli* cultures containing recombinant plasmid expressing these CHDLs, as described previously [17]. OXA-211, OXA-213 and OXA-214 showed a peculiar hydrolysis profile. These enzymes were able to hydrolyse penicillins and carbapenems. However, as observed for most of the CHDLs, broad-spectrum cephalosporins and monobactams were spared [20]. The rates of carbapenem hydrolysis were similar to those of OXA-23 and OXA-51 (Table 3).

Genetic context of OXA-211, OXA-213 and OXA-214 CHDL-encoding genes

In silico analysis of whole genome sequences of *A. johnsonii*, *A. haemolyticus* and *A. calcoaceticus* led to the identification of genes surrounding these CHDL-encoding genes. These genes were chromosomally located. Upstream of the $bla_{OXA-211}$ gene from *A. johnsonii*, the *dnaK* gene was identified, encoding a molecular chaperone that modulates the heat shock response in *E. coli.* Downstream of the $bla_{OXA-211}$ gene, there was a hypothetical open reading frame followed by a matrixin superfamily protein-encoding gene. Upstream of the $bla_{OXA-213}$ gene from *A. calcoaceticus*, a gene encoding a putative phage

TABLE 2. MICs of β-lactams for representative strains of *Acinetobacter* species and recombinant strains containing oxacillinase genes

	MIC (mg/L)										
β-Lactamases	*Acinetobacter baumannii* AYE	*Acinetobacter radioresistens* keb	*Acinetobacter calcoaceticus* CIP81.8	*Acinetobacter haemolyticus* Val	*Acinetobacter johnsonii* CIP70.16	*Escherichia coli* TOP10 (pTOPO-OXA-69)	*E. coli* TOP10 (pTOPO-OXA-23)	*E. coli* TOP10 (pTOPO-OXA-213)	*E. coli* TOP10 (pTOPO-OXA-214)	*E. coli* TOP10 (pTOPO-OXA-211)	*E. coli* TOP10
Amoxycillin	>256	32	2	8	8	16	>256	48	256	256	2
Amoxycillin + CLA	>256	8	1	2	4	8	256	48	128	128	2
Ticarcillin	>256	16	2	1	4	8	>256	48	128	128	2
Ticarcillin + CLA	32	8	1	0.5	2	8	128	48	128	128	2
Piperacillin	>256	8	1	6	64	4	16	6	8	6	1
Piperacillin + TZP	8	2	1	2	16	4	8	4	4	4	1
Cefotaxime	>256	1	0.12	4	32	0.06	0.06	0.06	0.06	0.06	0.06
Ceftazidime	>256	0.75	0.12	2	8	0.12	0.12	0.12	0.12	0.12	0.12
Cefepime	256	0.12	0.12	1	8	0.06	0.06	0.06	0.06	0.06	0.06
Aztreonam	>256	4	2	4	64	0.03	0.03	0.03	0.03	0.03	0.03
Meropenem	0.5	0.38	0.03	0.25	0.19	0.02	0.09	0.032	0.06	0.03	0.02
Doripenem	0.5	0.38	0.06	0.12	0.12	0.02	0.06	0.032	0.06	0.03	0.02
Imipenem	1	0.25	0.12	0.12	0.12	0.12	1	0.25	0.12	0.25	0.12
Ertapenem	8	3	0.06	1	3	0.06	0.12	0.06	0.12	0.06	0.06

CLA, clavulanic acid (4 mg/L); TZB, tazobactam (4 mg/L).

TABLE 3. Specific activities of OXA-51-like from *Acinetobacter baumannii*, OXA-23 from *Acinetobacter radioresistens* (used as reference), OXA-211 from *Acinetobacter johnsonii*, OXA-213 from *Acinetobacter calcoaceticus* and OXA-214 from *Acinetobacter haemolyticus* in *Escherichia coli* TOP10

Substrate (100 μM)	Specific activity (mU/mg)[a]				
	OXA-51-like	OXA-213	OXA-214	OXA-211	OXA-23
Benzylpenicillin	98.6	443.0	186.5	169.2	854.1
Cefotaxime	–	–	–	–	–
Ceftazidime	–	–	–	–	–
Aztreonam	–	–	–	–	–
Imipenem	0.41	3.1	2.1	2.7	4.5
Meropenem	0.35	0.1	–	0.1	0.9
Ertapenem	–	2.3	1.5	0.35	0.8

–, no detectable hydrolysis.
[a]Standard deviations were within 10%.

protein was identified, whereas a sortase family protein-encoding gene was identified downstream. Upstream of the $bla_{OXA-214}$ gene from *A. haemolyticus*, a gene encoding an outer membrane protein was identified, and a GGDEF family protein-encoding gene was identified downstream. This latter configuration has already been observed for the bla_{OXA-23} gene from *A. radioresistens*, which was identified upstream of an AAA ATPase family protein-encoding gene [10].

In conclusion, this study identified naturally occurring CHDL-encoding genes in *A. haemolyticus*, *A. johnsonii*, and *A. calcoaceticus*. None of them corresponded to the acquired CHDL-encoding genes reported in *A. baumannii* or in any other Gram-negative rods. As these genes remained highly specific to these species, detection of these genes might be used as an additional tool for rapid identification of these *Acinetobacter* species. Finally, this work further indicates that *Acinetobacter* species are the reservoirs of peculiar β-lactamase genes, actually CHDL genes, whose distribution seems to be restricted to Gram-negative rods (*Pseudomonas*, *Aeromonas*, etc.). Their natural function remains to be identified.

Nucleotide sequence accession numbers

The nucleotide sequences of the $bla_{OXA-211}$, $bla_{OXA-212}$, $bla_{OXA-213}$, $bla_{OXA-214}$ and $bla_{OXA-215}$ genes reported in this article are available in the GenBank nucleotide database under the following accession numbers: JN861779 for OXA-211; JN861780 for OXA-212; JN861781 for OXA-213; JN861782 for OXA-214; and JN861783 for OXA-215.

Transparency Declaration

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